

**AN EEG STUDY OF  
MORPHINE TOLERANCE IN THE RAT**


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FOREWORD  
(Nontechnical summary)

Morphine produces somnolence and analgesia when it is first administered to many species. However, with repeated doses, more and more morphine is needed to produce these effects. In other words, the animals develop tolerance to the drug. EEG recordings from different regions of the brain were obtained to see if drug tolerance develops earlier in some brain regions than others. No systematic pattern with regard to onset of drug tolerance was established. Reversal of tolerance to morphine was found in rats after destruction of the medial thalamus.

## ABSTRACT

Tolerance, manifested by a diminished EEG response at cortical and subcortical recording sites, was found in rats subjected to repeated systemic injections of morphine sulfate. Reversal of tolerance to morphine resulted from destruction of the medial thalamus.

## I. INTRODUCTION

After repeated administration of morphine, there comes a point when the same previously effective dose no longer has the desired effect. If the dosage is not increased, withdrawal symptoms frequently occur. In the rat, withdrawal is manifested by agitation, hyperreactivity to tactile and auditory stimuli, increased locomotor activity and "wet shakes". An excellent description of these withdrawal symptoms has been provided by Kerr and Pozuelo.<sup>3</sup>

Several hypotheses have been put forth to explain how drug tolerance develops.<sup>2</sup> It has generally been assumed that morphine tolerance is manifested throughout the nervous system in a nonspecific manner. Wikler and Carter<sup>8</sup> studied the depressant effects of repeated doses of morphine upon spinal reflexes in the caudal portion of the surgically sectioned spinal cord of the dog and were able to demonstrate the gradual appearance of drug tolerance, i.e., loss of effectiveness of the drug after repeated administration.

Berkowitz and Spector<sup>1</sup> demonstrated that it is possible to transfer tolerance from mice with a history of repeated morphine treatments to naive mice by injecting serum containing morphine immunogen. They suggested that these antibodies "reduced the concentration of morphine at critical sites in the brain." Recently, Pert and Snyder<sup>5</sup> have shown that opiates do not bind equally to all cells in the CNS. For example, the caudate nucleus seems to have a greater affinity to these drugs than the cerebral cortex or cerebellum.

Further evidence suggesting that opiates have a discrete locus of action in the brain was described by Wei et al.<sup>7</sup> In their experiments, precipitation of withdrawal



symptoms after morphine administration was produced by direct injections of minute amounts of naloxone (a potent antagonist) into the medial thalamus. Naloxone injected at many other subcortical sites had no effect.

Despite the recent biochemical evidence that opiates have a greater affinity for the basal ganglia of the brain than for other regions, there have been no reports of localized or selective bioelectrical changes in response to morphine injections. Changes in cortical electrical activity in response to morphine administration have been studied,<sup>4</sup> but bioelectrical assays of different regions of the brain have not been obtained to see if morphine responses can be observed in some brain regions before others. It is also possible that tolerance, manifested by a diminished EEG response to morphine, would show up in some regions of the brain earlier than in other regions. For these reasons, we have compared the bioelectrical response of the caudate nucleus, medial thalamus and cortex to repeated doses of morphine. The effect of medial thalamic ablation on naloxone withdrawal symptoms was also studied.

## II. METHODS AND RESULTS

Ten male Sprague-Dawley rats were anesthetized with sodium pentobarbital (50 mg/kg). Bipolar recording electrodes were implanted at caudate, medial thalamic and occipital cortical sites. The signals were first amplified by Tektronix FM 122 units with a band-pass setting of 0.2-50 Hz. A Beckman chart recorder was used to obtain tracings of the bioelectrical signals.

The recordings in Figure 1 were obtained from a rat that developed tolerance very rapidly. Those in Figure 2 were from an animal that required three times as much morphine to become tolerant. When the first morphine sulfate dose (30 mg/kg

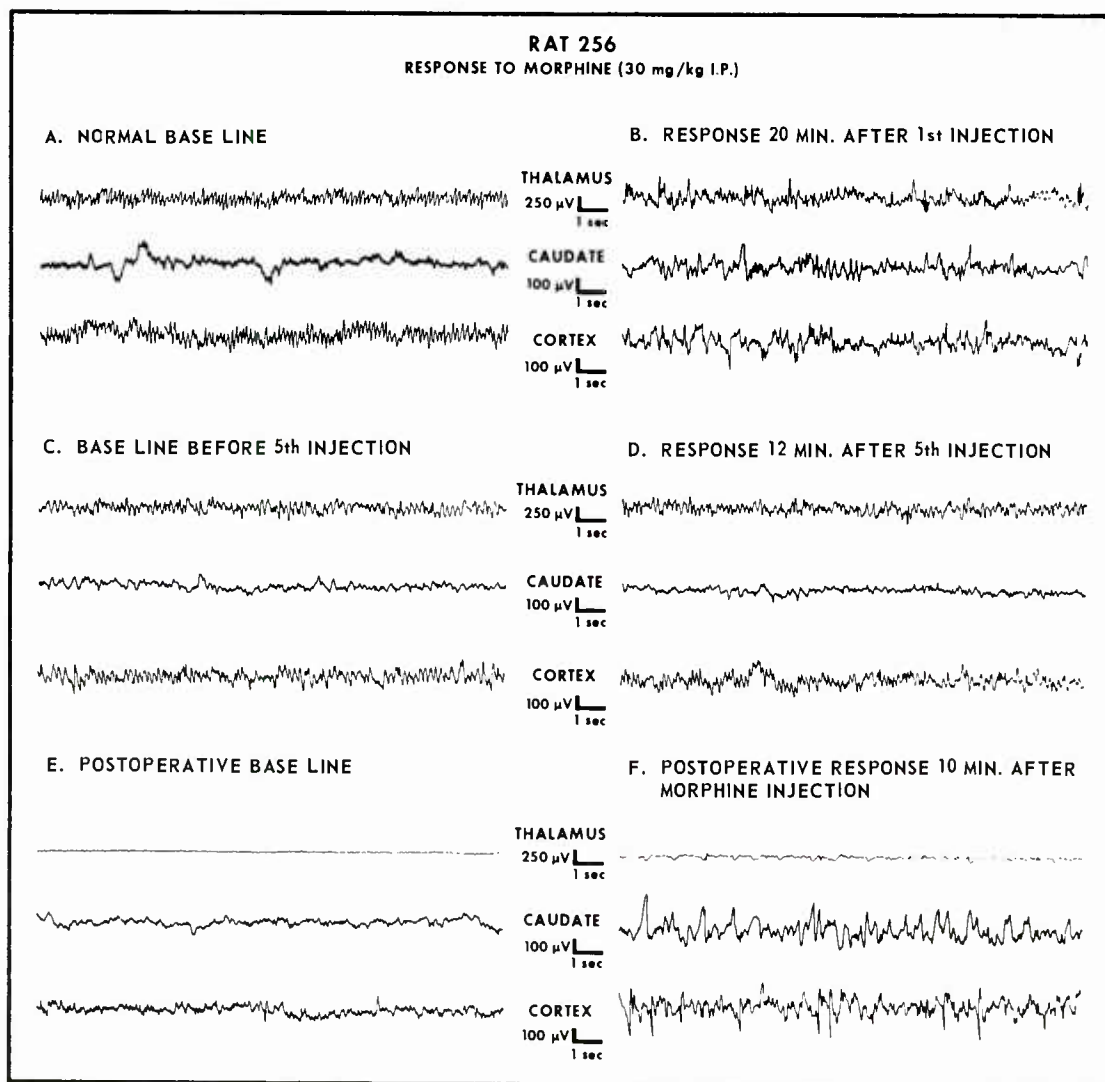
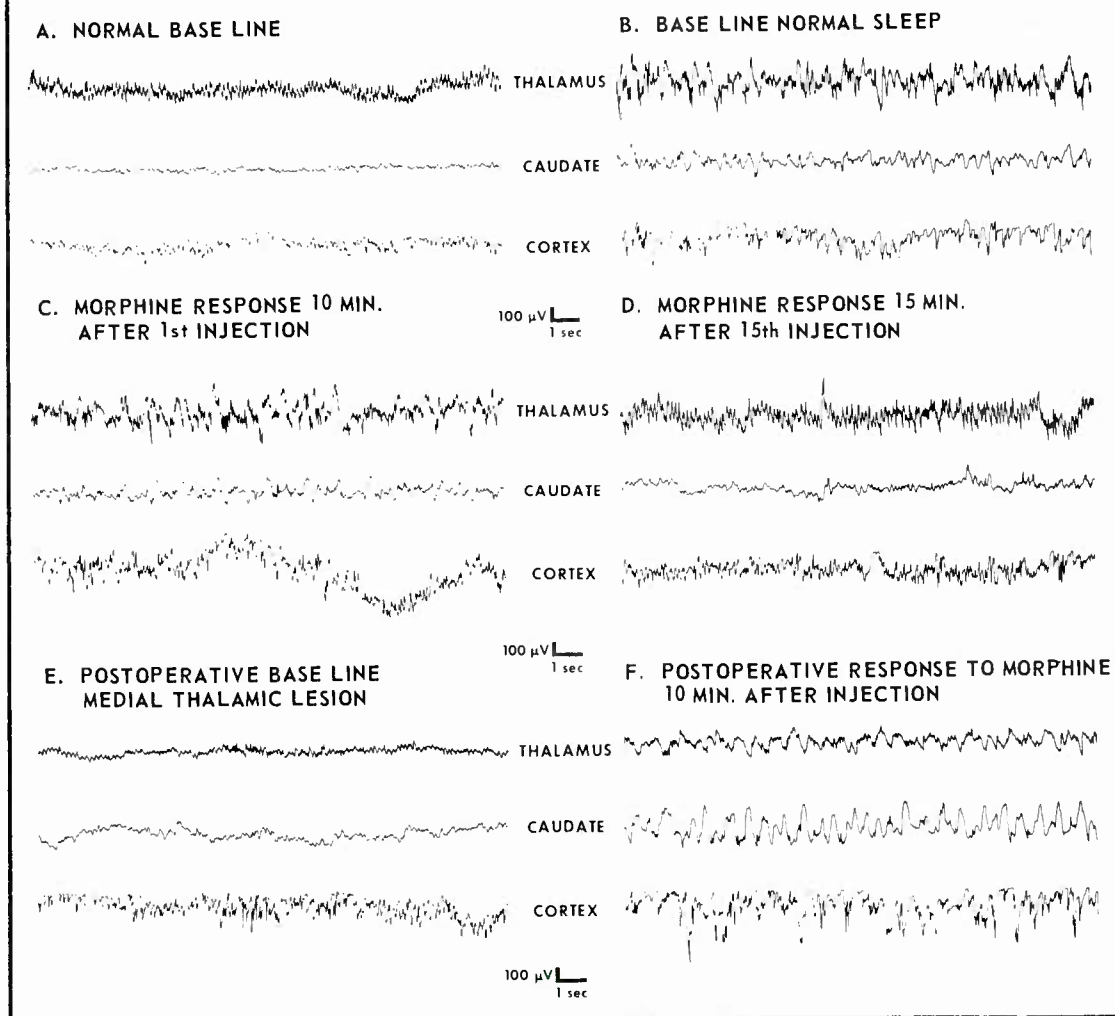


Figure 1. Effects of repeated injections of morphine sulfate upon EEG tracings recorded from thalamic (top trace), caudate nucleus (middle trace) and cortical (lower trace) sites. Tolerance to morphine is demonstrated in D. Medial thalamic lesion results in the reappearance of sensitivity to morphine (F).

intraperitoneally) was administered, the EEG patterns were drastically altered (Figures 1B and 2C). In some rats, the change occurred first at caudate recording sites, in others, the cortex showed high amplitude slow waves first. There was no predictable pattern from one subject to another.

**RAT 277**  
**RESPONSE TO MORPHINE (30 mg/kg I.P.)**



**Figure 2.** Effects of repeated injections of morphine sulfate upon EEG tracings recorded from thalamic (top trace), caudate nucleus (middle trace) and cortical (lower trace) sites. Compare normal sleep EEG (B) to morphine response (C). Tolerance to morphine is demonstrated in D. Reversal of morphine tolerance after medial thalamic lesion is seen in F.

EEG tracings obtained from normally sleeping subjects were almost identical to recordings obtained after morphine administration (Figure 2B, C). The onset of drug effect varied from 2.5 minutes to 15 minutes. The duration of the morphine effect

decreased gradually with each succeeding dose. By the fifth dose, rat 256 showed no behavioral response to morphine. After the injection, the rat remained alert and often showed abstinence signs characterized by head bobbing and wet shakes. The EEG (Figure 1D) remained the same as the base-line recordings (Figure 1C). Tolerance to morphine developed much more slowly in rat 277. By the 15th dose there was no EEG response to a single dose of morphine (Figure 2D). To obtain the pharmacological effect of the initial dose of morphine (Figures 1B, 2C), it was necessary to increase the dosage at least five times (150 mg/kg). This dosage would have been lethal if it were given as the initial dose.

After determining the dosage necessary to reinstitute the morphine effect in tolerant subjects, naloxone (0.8 mg/kg intraperitoneally) was administered. This caused a near immediate reversal of the morphine effect. That is, the rats became alert, the high amplitude slow waves were abolished and the EEG from all three recording sites reverted back to base line. The medial thalamic tracings did not lead the others as might be expected from the findings of Wei et al.<sup>7</sup> The naloxone effect lasted for about 15 minutes, then the rats became somnolent again.

To evaluate the ability of naloxone to reverse the effects of morphine when the medial thalamus was ablated, electrolytic destruction of this region was accomplished in four unanesthetized animals by passing .2 mA of dc current for 50 seconds. Lesions of the caudate nucleus and hippocampus were placed in six control subjects. Despite extensive damage to the medial and lateral habenular nuclei, the fasciculus retroflexus, the dentate gyrus and the nucleus medialis dorsalis (Figure 3), naloxone still reversed the effect of morphine. However, the duration of action of this morphine

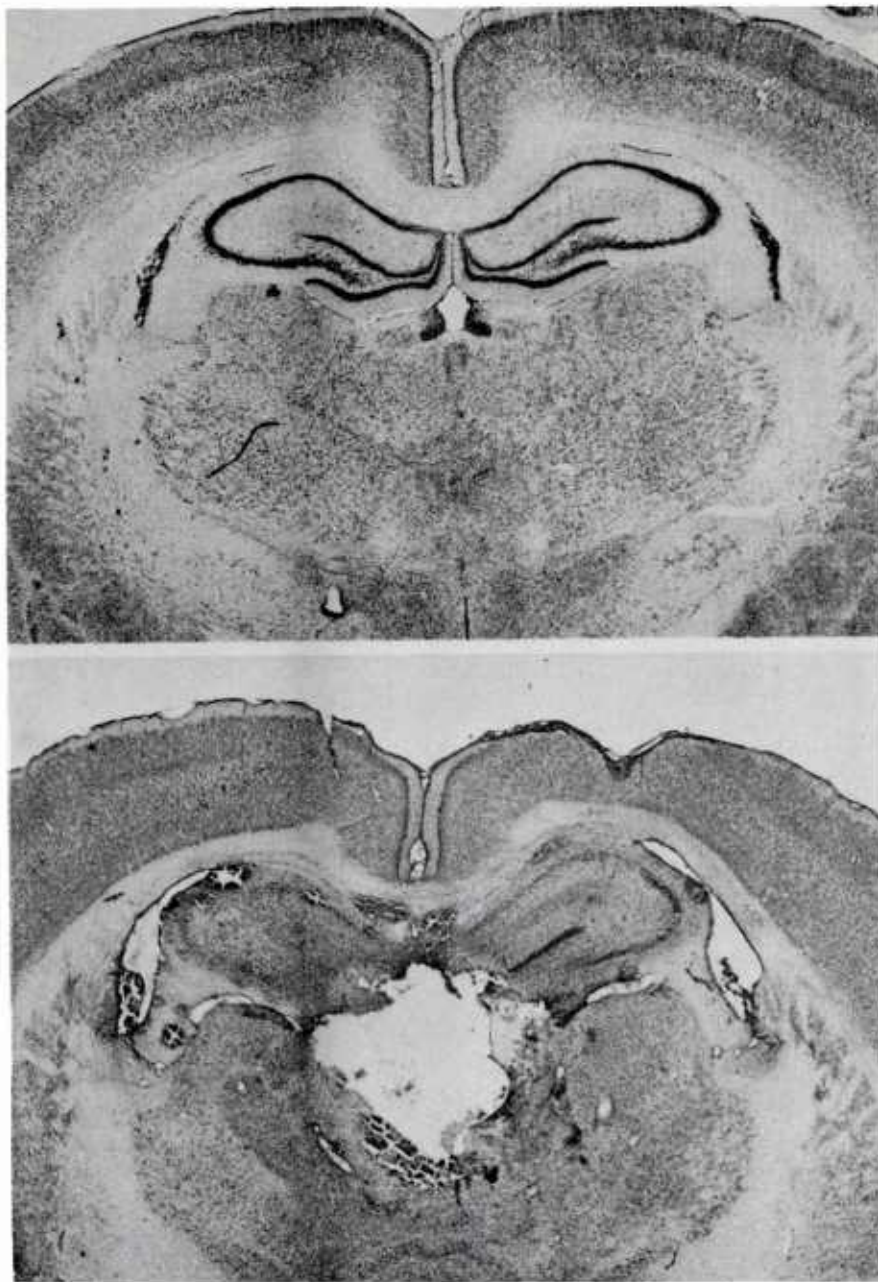


Figure 3. Photomicrographs of thionine stained frontal sections of the rat brain showing normal thalamus (top) and medial thalamic lesion (bottom). This lesion was effective in reversing morphine tolerance in the rat.



antagonist was slightly shorter in subjects with medial thalamic damage. Lesions of the caudate nucleus and dorsal hippocampus had no effect. The following day, EEG recordings obtained from caudate and cortical sites were normal (Figures 1E, 2E). The tracing from the medial thalamic site of subject 256 was completely flat as a result of the lesion. In subject 277 the medial thalamic tracing was drastically reduced in amplitude but not completely abolished. As soon as a single dose of morphine was administered, the EEG from the two intact recording sites showed an intense morphine response, and those subjects with medial thalamic ablations became somnolent (Figures 1F and 2F).

### III. DISCUSSION

While medial thalamic lesions were found to have little if any effect upon precipitated withdrawal produced by naloxone, these lesions did have a drastic effect upon sensitivity to morphine in tolerant rats. According to Wei et al.,<sup>7</sup> intracerebral injections of naloxone crystals were effective only when injected into the medial thalamic region. Thus, it is somewhat surprising that systemic injections of this morphine antagonist are still effective when this region is ablated. It is possible, despite the conclusion by Wei et al.,<sup>7</sup> that the medial thalamus is not the only site where naloxone acts, and there still remain other loci that respond to naloxone in the absence of the medial thalamus. The medial thalamic ablations included damage to the habenular complex, stria medullaris and fasciculus retroflexus, as well as damage to the nucleus medialis dorsalis. It is therefore unlikely that our lesions did not destroy enough of the critical medial thalamic zone described by Wei et al.<sup>7</sup> The finding that localized brain damage can affect morphine tolerance is not easily reconciled with theories that

tolerance to morphine is a generalized phenomenon occurring throughout the central nervous system. A similar effect has been demonstrated by Kerr and Pozuelo.<sup>3</sup> They showed that lesions of the ventromedial nucleus of the hypothalamus and, to a lesser extent, lesions of the septal nucleus of tolerant rats result in increased sensitivity to morphine. Since behavioral criteria were used in their experiments to assess tolerance, whereas EEG criteria were used in the present study, it is not possible at the present time to compare the magnitude of the effects of lesions of the medial thalamus to ablation of the septal region and hypothalamus. All three regions are related by fibers of the stria medullaris, a massive fiber tract capping the habenular nuclei. It is imprudent at this time to offer a neuroanatomical hypothesis to explain this drug-lesion interaction. It is possible that neurochemical alterations occurring in denervated cells in other regions of the brain are responsible for this altered sensitivity to morphine. This kind of effect has been described by Roth and Harvey<sup>6</sup> who showed that cells in the cerebral cortex are more sensitive to barbiturates after damage to the septal nucleus.

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